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=> d his

     (FILE 'HOME' ENTERED AT 14:23:15 ON 23 FEB 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:23:47 ON 23 FEB 2006
L1        395086 S BACILLUS
L2           560 S CRYIIIA
L3           516 S L1 AND L2
L4           635 S "TTGACA" OR "TATAAT"
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             7 S L2 AND "CONSENSUS PROMOTER"
L8             5 DUP REM L7 (2 DUPLICATES REMOVED)
                 E WIDNER W/AU
L9           119 S E3-E9
                 E SLOMA A/AU
L10          124 S E3
                 E THOMAS M D/AU
L11          404 S E3
L12          632 S L9 OR L10 OR L11
L13            8 S L2 AND L12
L14            6 DUP REM L13 (2 DUPLICATES REMOVED)
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 NEWS  7  DEC 21   IPC search and display fields enhanced in CA/CAplus with the
                   IPC reform
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                   USPAT2
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                   INPADOC
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 NEWS 13  JAN 30   Saved answer limit increased
 NEWS 14  JAN 31   Monthly current-awareness alert (SDI) frequency
                   added to TULSA
 NEWS 15  FEB 21   STN AnaVist, Version 1.1, lets you share your STN AnaVist
                   visualization results
 NEWS 16  FEB 22   Status of current WO (PCT) information on STN
 NEWS 17  FEB 22   The IPC thesaurus added to additional patent databases on STN
 NEWS 18  FEB 22   Updates in EPFULL; IPC 8 enhancements added

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=> s bacillus
L1      395086 BACILLUS

=> s cryIIIA
L2         560 CRYIIIA

=> s l1 and l2
L3         516 L1 AND L2

=> s "TTGACA" or "TATAAT"
L4         635 "TTGACA" OR "TATAAT"

=> s l3 and l4
L5          13 L3 AND L4

=> dup rem l5
PROCESSING COMPLETED FOR L5
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L6    ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2005-30339  BIOTECHDS
TITLE:            Producing a hyaluronic acid, by cultivating Bacillus
                  cell comprising a nucleic acid construct comprising a variant
                  amyL promoter, a consensus promoter, and a cryIIIA
                  promoter, and isolating hyaluronic acid from the cultivation
                  medium;
                     production of recombinant hyaluronic acid from a
```

Bacillus having a triple promoter useful for a tissue engineering application

AUTHOR: WIDNER W; SLOMA A; THOMAS M; TANG M
PATENT ASSIGNEE: NOVOZYMES BIOPOLYMER AS
PATENT INFO: US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO: US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-722702 [74]

AB DERWENT ABSTRACT:

NOVELTY - Producing a hyaluronic acid comprises cultivating a **Bacillus** cell in a medium for the production of the hyaluronic acid, where the **Bacillus** cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a consensus promoter, and a **cryIIIA** promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a **Bacillus** cell in a medium for the production of the hyaluronic acid, where the **Bacillus** cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a **Bacillus** cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a **Bacillus** cell; and(3) a selectable marker-free mutant of a **Bacillus** cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a **Bacillus** host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the **Bacillus** amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl

transferase gene. Producing a selectable marker-free mutant of a **Bacillus** cell comprises deleting a selectable marker gene of the **Bacillus** cell. The **Bacillus** cell contains no foreign selectable marker gene.

USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L6 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from **cryIIIa**-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region, and/or which is derived from amyL, amyQ, amyM, **cryIIIA**,

dagA, aprH, penP, sacB, spoI, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is **Bacillus** subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the **Bacillus** subtilis genome or in the **cryIIIa** promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a **Bacillus** host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L6 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), **Bacillus** clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes,

B. thuringiensis subsp. tenebrionis **CryIIIA** gene (**cryIIIA**) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the **cryIIIA** mRNA processing/stabilizing sequence. The **bacillus** cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence **TTGACA** for the 35 region, and **TATAAT** for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a **bacillus** promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L6 ANSWER 4 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a **Bacillus** cell in a medium conducive to the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the

cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a **Bacillus** cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a **Bacillus** host cell by introducing into a **Bacillus** cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a **Bacillus** cell by deleting a selectable marker gene of the **Bacillus** cell; and (4) a selectable marker-free mutant of a **Bacillus** cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more **Bacillus** genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** from the -10 region, the amyL promoter, and/or the **cryIIIA** promoter. The tandem promoter comprises two copies of the amyQ, amyl or **cryIIIA** promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the **Bacillus** cell. The mRNA processing/stabilizing sequence is the **cryIIIA** or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The **Bacillus** cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the **Bacillus** cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the **Bacillus** cell. The **Bacillus** host cell is **Bacillus** alkalophilus, **Bacillus** amyloliquefaciens, **Bacillus** brevis, **Bacillus** brevis, **Bacillus** circulans, **Bacillus** clausii, **Bacillus** coagulans, **Bacillus** firmus, **Bacillus** lautus, **Bacillus** lentus, **Bacillus** licheniformis, **Bacillus** megaterium, **Bacillus** pumilus, **Bacillus** sterothermophilus, **Bacillus** subtilis, or **Bacillus** thuringiensis. This method alternatively comprises cultivating a **Bacillus** cell in a medium conducive for the production of the polypeptide, where the **Bacillus** cell comprises a nucleic acid construct comprising a consensus promoter having the sequence **TTGACA** for the -35 region and **TATAAT** for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a **Bacillus**

promoter. Preferred Cell: The **Bacillus** cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a **Bacillus** cell, and for producing a selectable marker-free mutant of a **Bacillus** cell.

EXAMPLE - No relevant example given.(57 pages)

L6 ANSWER 5 OF 6 MEDLINE on STN DUPLICATE 3
ACCESSION NUMBER: 2001393416 MEDLINE
DOCUMENT NUMBER: PubMed ID: 11234961
TITLE: Construction of protein overproducer strains in **Bacillus** subtilis by an integrative approach.
AUTHOR: Jan J; Valle F; Bolivar F; Merino E.
CORPORATE SOURCE: Departamento de Microbiologia Molecular, Instituto de Biotecnologia, Universidad Nacional Autonoma de Mexico, Cuernavaca, Morelos.
SOURCE: Applied microbiology and biotechnology, (2001 Jan) 55 (1) 69-75.
Journal code: 8406612. ISSN: 0175-7598.
PUB. COUNTRY: Germany: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200107
ENTRY DATE: Entered STN: 20010716
Last Updated on STN: 20010716
Entered Medline: 20010712

AB We evaluated the effect of several genetic factors reported as having a role in the induction of the expression of significant levels of recombinant protein in **Bacillus** subtilis. We utilized the beta-galactosidase reporter protein from Escherichia coli as our model for measuring the overproduction of heterologous proteins in B. subtilis. The lacZ gene was expressed in B. subtilis using the regulatory region of the subtilisin gene aprE. In this study, we considered factors known to modulate the transcription and translation initiation rates and genetic and mRNA stability. We also consider the effects of different genetic backgrounds, such as degU32 and hpr2, that until now have been studied independently. By changing the native -35 promoter box to the consensus **TTGACA** sequence of the aprE promoter, a significant 100-fold increase in the beta-galactosidase activity was obtained. On the other hand, changes such as the GTG to ATG start codon, the construction of a consensus AAGGAGG ribosome binding site, and the addition of the **cryIIIA** transcription terminator at the 3' end of the lacZ gene, produced only marginal effects on the final beta-galactosidase activity.

L6 ANSWER 6 OF 6 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a **Bacillus** cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		

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            SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD,
            RU, TJ, TM
        RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK,
            ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG,
            CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG
    US 5955310          A     19990921    US 1998-31442          19980226
    AU 9929756          A1    19990915    AU 1999-29756          19990226
    EP 1056873          A2    20001206    EP 1999-911012         19990226
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                                            WO 1999-US4360      W  19990226
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AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a **Bacillus** host cell in a medium conducive for the production of the polypeptide, wherein the **Bacillus** cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence **TTGACA** for the "-35" region and **TATAAT** for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the **cryIIIA** promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the **cryIIIA** mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in **Bacillus** cells when compared to the levels obtained using single promoters such as amyQ and amyL.

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     (FILE 'HOME' ENTERED AT 14:23:15 ON 23 FEB 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:23:47 ON 23 FEB 2006
L1        395086 S BACILLUS
L2           560 S CRYIIIA
L3           516 S L1 AND L2
L4           635 S "TTGACA" OR "TATAAT"
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)

=> s l2 and "consensus promoter"
L7             7 L2 AND "CONSENSUS PROMOTER"

=> dup rem l7
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PROCESSING COMPLETED FOR L7
L8 5 DUP REM L7 (2 DUPLICATES REMOVED)

=> d 1-5 ibib ab

L8 ANSWER 1 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2005-30339 BIOTECHDS
TITLE: Producing a hyaluronic acid, by cultivating Bacillus cell comprising a nucleic acid construct comprising a variant amyL promoter, a **consensus promoter**, and a **cryIIIA** promoter, and isolating hyaluronic acid from the cultivation medium;
production of recombinant hyaluronic acid from a Bacillus having a triple promoter useful for a tissue engineering application
AUTHOR: WIDNER W; SLOMA A; THOMAS M; TANG M
PATENT ASSIGNEE: NOVOZYMES BIOPOLYMER AS
PATENT INFO: US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO: US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-722702 [74]
AB DERWENT ABSTRACT:
NOVELTY - Producing a hyaluronic acid comprises cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a **consensus promoter**, and a **cryIIIA** promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a **consensus promoter** having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a Bacillus cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a **consensus promoter** having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a Bacillus cell; and(3) a selectable marker-free mutant of a Bacillus cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a Bacillus host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a **consensus promoter** having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid,

the variant amyL promoter is SEQ ID NO. 1. The **consensus promoter** is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign selectable marker gene.

USE - The method is useful for producing a hyaluronic acid. Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L8 ANSWER 2 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 1
ACCESSION NUMBER: 2004-02033 BIOTECHDS
TITLE: Generating an expression library of polynucleotides by introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest;
protein library screening using homologous recombination
AUTHOR: BJORNVAD M E; JORGENSEN P L; HANSEN P K
PATENT ASSIGNEE: NOVOZYMES AS
PATENT INFO: WO 2003095658 20 Nov 2003
APPLICATION INFO: WO 2003-DK301 7 May 2003
PRIORITY INFO: DK 2002-682 7 May 2002; DK 2002-682 7 May 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
AB DERWENT ABSTRACT:

NOVELTY - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest.

DETAILED DESCRIPTION - Generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell comprises: (a) providing a non-replicating linear integration cassette; and (b) introducing the linear integration cassette into the host cell and selecting or screening for host cells that produce the polypeptides of interest. The cassette comprises: (1) a polynucleotide encoding one or more polypeptides of interest; (2) a 5' flanking polynucleotide segment upstream of the polynucleotide of (1) and comprising a first homologous region located in the 3' end of the segment; and (3) a 3' flanking polynucleotide segment downstream of the polynucleotide of (1) and comprising a second homologous region located in the 5' end of the segment. The first and second homologous regions are at least 500, 1000 or 1500 bp, each of which has a sequence identity of at least 80, 85, 90 or 95-100% with a region of the host cell genom. INDEPENDENT CLAIMS are also included for the following: (1) a non-replicating linear Gram-positive host cell integration cassette; and (2) a method of producing a polypeptide of interest.

BIOTECHNOLOGY - Preferred Method: Generating an expression library of polynucleotides integrated by homologous recombination into the genome

of a competent Gram-positive bacterium host cell comprises the additional step carried out between steps (1) and (2) that comprises introducing the plasmid into an intermediate Escherichia coli host cell and propagating it by replication. The integration cassette comprises: (1) an mRNA processing/stabilizing sequence derived from **cryIIIa**-gene; (2) a terminator downstream of the polynucleotide encoding the polypeptides of interest; and (3) a multiple cloning site with at least one recognition site for a restriction nuclease. It further comprises a marker gene located between the 5' and 3' flanking segments and at least one promoter that is a **consensus promoter** having the sequence TTGACA for the -35 region and TATAAT for the -10 region, and/or which is derived from amyL, amyQ, amyM, **cryIIIA**, dagA, aprH, penP, sacB, spol, tac, xylA or xylB. The promoter is located between the flanking segments and is operably linked to the polynucleotide encoding one or more polypeptides of interest. Each of the 5' and 3' flanking polynucleotide segments comprises at least 500, 1000, 1500 or 2000 bp of non-homologous polynucleotides located in the 5' and 3' end of the 5' and 3' flanking segments, respectively. The promoter is one that results in that the host cells produce the polypeptides of interest in a yield of at least 10 mg/L. The polynucleotide comprises natural, synthetic or a library of shuffled or recombined homologs or variants of a gene or operon, provided by DNA breeding or DNA shuffling. The polypeptides of interest comprise enzymes, proteins or antimicrobial peptides. The enzymes are involved in the biosynthesis of hyaluronic acid. The Gram-positive host cell is Bacillus subtilis. The homologous region of the 5' and/or the 3' flanking segment is comprised in the yfmD-yfmC-yfmB-yfmA-pelB-yflS-citS region of the Bacillus subtilis genome or in the **cryIIIa** promoter. The non-replicating linear integration cassette is comprised in a plasmid and introduced into the host cell. The plasmid is capable of replicating in an Escherichia coli host cell but not in a Bacillus host cell. Producing a polypeptide of interest comprises culturing Gram-positive host cells comprising the integration cassette integrated into its genome, under conditions promoting expression of the polypeptide of interest. The method further comprises isolating and/or purifying the polypeptide of interest.

USE - The method is useful in generating an expression library of polynucleotides integrated by homologous recombination into the genome of a competent Gram-positive bacterium host cell (claimed). (55 pages)

L8 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2

ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: THOMAS M D; SLOMA A
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]

AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The **consensus promoter** is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis **CryIIIA** gene (**cryIIIA**) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the **cryIIIA** mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a **consensus promoter** having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the **consensus promoter**, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The **consensus promoter** is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L8 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: WIDNER W; SLOMA A; THOMAS M D
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003

APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]

AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a **consensus promoter** having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the **cryIIIA** promoter. The tandem promoter comprises two copies of the amyQ, amyl or **cryIIIA** promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the **cryIIIA** or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a **consensus promoter** having the sequence TTGACA for the -35 region and TATAAT

for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the **consensus promoter** and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The **consensus promoter** is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L8 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2006 ACS on STN
ACCESSION NUMBER: 1999:566201 HCAPLUS
DOCUMENT NUMBER: 131:180803
TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell
INVENTOR(S): Widner, William; Sloma, Alan; Thomas, Michael D.
PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA
SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 3
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
CN 1510145	A	20040707	CN 2003-2003158121	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412
PRIORITY APPLN. INFO.:			US 1998-31442 A	19980226
			US 1999-256377 B3	19990224
			WO 1999-US4360 W	19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a " **consensus" promoter** having the sequence TTGACA for the

"-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "**consensus**" **promoter** and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the **cryIIIA** promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the **cryIIIA** mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:23:47 ON 23 FEB 2006
L1        395086 S BACILLUS
L2           560 S CRYIIIA
L3           516 S L1 AND L2
L4           635 S "TTGACA" OR "TATAAT"
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             7 S L2 AND "CONSENSUS PROMOTER"
L8             5 DUP REM L7 (2 DUPLICATES REMOVED)

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E2            2     WIDNER THOMAS E/AU
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E5           43     WIDNER W R/AU
E6           14     WIDNER WILLIAM/AU
E7           22     WIDNER WILLIAM R/AU
E8            1     WIDNER WILLIAM ROY/AU
E9            1     WIDNER WM R/AU
E10           5     WIDNES C/AU
E11           2     WIDNES J/AU
E12           2     WIDNES J A/AU

=> s e3-e9
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                OR "WIDNER WM R"/AU)

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E5           60     SLOMA ALAN/AU
E6           16     SLOMA ALAN P/AU
E7            1     SLOMA ALAN PAUL/AU
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E9            4     SLOMA CARI R/AU
E10           5     SLOMA CARI ROARK/AU
E11           1     SLOMA D/AU
E12           1     SLOMA D R/AU
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L10         124 "SLOMA A"/AU

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E4          105     THOMAS M D A/AU
E5            4     THOMAS M D H/AU
E6            3     THOMAS M D JR/AU
E7            2     THOMAS M D O/AU
E8           17     THOMAS M D R/AU
E9          346     THOMAS M E/AU
E10          33     THOMAS M E A/AU
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=> d his

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     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:23:47 ON 23 FEB 2006
L1        395086 S BACILLUS
L2           560 S CRYIIIA
L3           516 S L1 AND L2
L4           635 S "TTGACA" OR "TATAAT"
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             7 S L2 AND "CONSENSUS PROMOTER"
L8             5 DUP REM L7 (2 DUPLICATES REMOVED)
                 E WIDNER W/AU
L9           119 S E3-E9
                 E SLOMA A/AU
L10          124 S E3
                 E THOMAS M D/AU
L11          404 S E3

=> s l9 or l10 or l11
L12          632 L9 OR L10 OR L11

=> s l2 and l12
L13            8 L2 AND L12

=> dup rem l13
PROCESSING COMPLETED FOR L13
L14            6 DUP REM L13 (2 DUPLICATES REMOVED)

=> d 1-6 ibib ab

L14   ANSWER 1 OF 6  BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
      DUPLICATE 1
ACCESSION NUMBER: 2005-30339  BIOTECHDS
TITLE:            Producing a hyaluronic acid, by cultivating Bacillus cell
                  comprising a nucleic acid construct comprising a variant amyL
                  promoter, a consensus promoter, and a cryIIIA
                  promoter, and isolating hyaluronic acid from the cultivation
                  medium;
                     production of recombinant hyaluronic acid from a Bacillus
                     having a triple promoter useful for a tissue engineering
                     application
```

AUTHOR: **WIDNER W; SLOMA A**; THOMAS M; TANG M
PATENT ASSIGNEE: NOVOZYMES BIOPOLYMER AS
PATENT INFO: US 2005221446 6 Oct 2005
APPLICATION INFO: US 2005-96190 31 Mar 2005
PRIORITY INFO: US 2005-96190 31 Mar 2005; US 2004-558507 31 Mar 2004
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2005-722702 [74]

AB DERWENT ABSTRACT:

NOVELTY - Producing a hyaluronic acid comprises cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter, a consensus promoter, and a **cryIIIA** promoter, and isolating the hyaluronic acid from the cultivation medium.

DETAILED DESCRIPTION - Producing a hyaluronic acid comprises: (A) cultivating a Bacillus cell in a medium for the production of the hyaluronic acid, where the Bacillus cell comprises a nucleic acid construct comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of a fully defined 614 bp sequence (SEQ ID NO. 1), a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of the hyaluronic acid; and (B) isolating the hyaluronic acid from the cultivation medium. INDEPENDENT CLAIMS are also included for: (1) a Bacillus cell comprising a nucleic acid construct which comprises: (a) a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid, and optionally (b) an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid; (2) producing a selectable marker-free mutant of a Bacillus cell; and(3) a selectable marker-free mutant of a Bacillus cell obtained by the method above.

WIDER DISCLOSURE - Also disclosed are: (1) methods for obtaining a Bacillus host cell; and (2) nucleic acid constructs comprising a triple promoter comprising a variant amyL promoter having a mutation corresponding to position 590 of SEQ ID NO. 1, a consensus promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region, and a **cryIIIA** promoter, in which each promoter sequence of the triple promoter is operably linked to one or more coding sequences involved in the biosynthesis of a hyaluronic acid.

BIOTECHNOLOGY - Preferred Method: In producing a hyaluronic acid, the variant amyL promoter is SEQ ID NO. 1. The consensus promoter is obtained from the Bacillus amyloliquefaciens alpha-amylase gene (amyQ), where the consensus amyQ promoter has the nucleotide sequence comprising fully defined 185 bp sequence (SEQ ID NO. 42 or 43). The nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the triple promoter and upstream of the one or more coding sequences involved in the biosynthesis of the hyaluronic acid. Preferably, the one or more coding sequences involved in the biosynthesis of the hyaluronic acid are selected from a hyaluronan synthase, UDP-glucose 6-dehydrogenase, UDP-glucose pyrophosphorylase, UDP-N-acetylglucosamine pyrophosphorylase, glucose-6-phosphate isomerase, hexokinase, phosphoglucomutase, amidotransferase, mutase, or acetyl transferase gene. Producing a selectable marker-free mutant of a Bacillus cell comprises deleting a selectable marker gene of the Bacillus cell. The Bacillus cell contains no foreign selectable marker gene.

USE - The method is useful for producing a hyaluronic acid.

Hyaluronic acid is useful in eye and joint surgery. Products of hyaluronic acid are also useful in orthopedics, rheumatology, and in dermatology.

EXAMPLE - No relevant example given.(94 pages)

L14 ANSWER 2 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-08483 BIOTECHDS
TITLE: Production of a secreted polypeptide having L-asparaginase activity for treating leukemia, comprises cultivating a host cell comprising a nucleic acid having a sequence encoding a secretory signal peptide linked to a second sequence; vector-mediated enzyme gene transfer and expression in host cell for recombinant protein production, amino acid preparation and disease therapy
AUTHOR: **THOMAS M D; SLOMA A**
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003186380 2 Oct 2003
APPLICATION INFO: US 2003-406025 1 Apr 2003
PRIORITY INFO: US 2003-406025 1 Apr 2003; US 2002-369192 1 Apr 2002
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2004-088916 [09]
AB DERWENT ABSTRACT:

NOVELTY - Producing a secreted polypeptide having L-asparaginase activity, comprises cultivating a host cell containing a nucleic acid construct having a sequence encoding a secretory signal peptide linked to a second sequence encoding the polypeptide having L-asparaginase activity, where the signal peptide directs the polypeptide into the cell's secretory pathway, and recovering the secreted polypeptide.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is also included for a recombinant expression vector comprising the nucleic acid construct.

BIOTECHNOLOGY - Preferred Material: The polypeptide is encoded by a nucleic acid sequence contained in plasmid pCR2.1-yccC, which is contained in Escherichia coli (NRRL B-30558). The consensus promoter is obtained from a promoter obtained from the E. coli lac operon Streptomyces coelicolor agarase gene (dagA), Bacillus clausii alkaline protease gene (aprH), B. licheniformis alkaline protease gene (subtilisin Carlsberg gene), B. subtilis levansucrase gene (sacB), B. subtilis alpha-amylase gene (amyE), B. licheniformis alpha-amylase gene (amyL), B. stearothermophilus maltogenic amylase gene (amyM), B. licheniformis penicillinase gene (penP), B. subtilis xylA and xylB genes, B. thuringiensis subsp. tenebrionis **CryIIIA** gene (**cryIIIA**) or its portions, or preferably B. amyloliquefaciens alpha-amylase gene (amyQ). The mRNA processing/stabilizing sequence is the **cryIIIA** mRNA processing/stabilizing sequence. The bacillus cell is B. alkalophilus, B. amyloliqifaciens, B. brevis, B. circulans, B. claussi, B. coagulans, B. lautus, B. lentus, B. licheniformis, B. megaterium, B. stearothermophilus, B. subtilis, or B. thuringiensis. Preferred Component: The nucleic acid construct comprises a tandem promoter, in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing stabilizing sequence located downstream of the tandem promoter and upstream of the second nucleic acid sequence, encoding the polypeptide having L-asparaginase activity. It comprises a consensus promoter having the sequence TTGACA for the 35 region, and TATAAT for the 10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing stabilizing sequence located downstream of the consensus promoter, and upstream of the second nucleic acid sequence encoding the polypeptide having L-asparagine activity. The consensus promoter is obtained from any bacterial or a bacillus promoter. The nucleic acid construct comprises a ribosome binding site sequence heterologous to the host cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - L-asparaginase.

USE - The invention is for production of secreted polypeptide having L-asparaginase activity, for use in producing L-aspartate from L-asparagine. The inventive secreted polypeptide is useful for treatment of leukemia, e.g. acute lymphocytic leukemia.

ADVANTAGE - The invention achieves secretion of L-asparaginase enabling easy recovery and purification, high expression constructs for producing the L-asparaginase in high amounts, and the use of host cells for production that have generally regarded as safe status.

EXAMPLE - B. subtilis strains MDT51 and MDT52 were grown in Lactobacilli MRS Broth (RTM; 50 ml) at 37degreesC, and 250 revolutions/minute (rpm) for 24 hours. Supernatants were recovered by centrifugation at 7000 rpm for 5 minutes. A prominent band corresponding to a protein of the expected size for mature L-asparagine (37 kDa; amino acids 24-375) was observed in the MDT51 sample, but not in the MDT52 sample. (22 pages)

L14 ANSWER 3 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
ACCESSION NUMBER: 2004-04169 BIOTECHDS
TITLE: Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter;
involving vector-mediated gene transfer and expression in host cell for use as a selectable marker
AUTHOR: **WIDNER W; SLOMA A; THOMAS M D**
PATENT ASSIGNEE: NOVOZYMES BIOTECH INC
PATENT INFO: US 2003170876 11 Sep 2003
APPLICATION INFO: US 2001-834271 12 Apr 2001
PRIORITY INFO: US 2001-834271 12 Apr 2001; US 1998-31442 26 Feb 1998
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 2003-898275 [82]
AB DERWENT ABSTRACT:

NOVELTY - Producing a polypeptide comprises cultivating a Bacillus cell in a medium conducive to the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a nucleic acid sequence encoding the polypeptide, and isolating the polypeptide from the cultivation medium.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the following: (1) a Bacillus cell comprising a nucleic acid construct comprising a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding a polypeptide, and optionally an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; (2) a method for obtaining a Bacillus host cell by introducing into a Bacillus cell the nucleic acid construct cited above; (3) a method for producing a selectable marker-free mutant of a Bacillus cell by deleting a selectable marker gene of the Bacillus cell; and (4) a selectable marker-free mutant of a Bacillus cell.

BIOTECHNOLOGY - Preferred Method: In producing a polypeptide, the nucleic acid construct further comprises an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide. The tandem promoter comprises two or more bacterial promoter sequences, which are obtained from one or more Bacillus genes. The tandem promoter comprises the amyQ promoter, a consensus promoter having the sequence TTGACA for the -35 region and TATAAT from the -10 region, the amyL promoter, and/or the **cryIIIA** promoter. The tandem promoter comprises two copies of the amyQ, amyl or **cryIIIA** promoter. The two or more promoter sequences of the tandem promoter simultaneously promote the transcription of the nucleic acid sequence. The one or more of the two or more promoter sequences of the tandem promoter promote the transcription of the nucleic

acid sequence at different stages of growth of the Bacillus cell. The mRNA processing/stabilizing sequence is the **cryIIIA** or SP82 mRNA processing/stabilizing sequence, which generates mRNA transcripts essentially of the same size. The Bacillus cell contains one or more copies of the nucleic acid construct. The nucleic acid construct further comprises a selectable marker gene. The nucleic acid sequence encodes a polypeptide heterologous to the Bacillus cell. The polypeptide is a hormone or its variant, enzyme, receptor or its portion, antibody or its portion, or reporter. The enzyme is an oxidoreductase, transferase, hydrolase, lyase, isomerase or ligase. The enzyme is preferably an aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, invertase, laccase, lipase, mannosidase, mutanase, oxidase, a pectinolytic enzyme, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase or xylanase. The nucleic acid sequence is contained in the chromosome of the Bacillus cell. The Bacillus host cell is Bacillus alkalophilus, Bacillus amyloliquefaciens, Bacillus brevis, Bacillus brevis, Bacillus circulans, Bacillus clausii, Bacillus coagulans, Bacillus firmus, Bacillus lautus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus pumilus, Bacillus sterothermophilus, Bacillus subtilis, or Bacillus thuringiensis. This method alternatively comprises cultivating a Bacillus cell in a medium conducive for the production of the polypeptide, where the Bacillus cell comprises a nucleic acid construct comprising a consensus promoter having the sequence TTGACA for the -35 region and TATAAT for the -10 region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and an mRNA processing/stabilizing sequence located downstream of the consensus promoter and upstream of the nucleic acid sequence encoding the polypeptide; and isolating the polypeptide from the cultivation medium. The consensus promoter is obtained from any bacterial promoter, preferably a Bacillus promoter. Preferred Cell: The Bacillus cell comprises a nucleic acid construct that further comprises a selectable marker gene. The cell can also contain no selectable marker gene.

USE - The methods are useful for producing a polypeptide in a Bacillus cell, and for producing a selectable marker-free mutant of a Bacillus cell.

EXAMPLE - No relevant example given.(57 pages)

L14 ANSWER 4 OF 6 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 2001:630833 HCAPLUS
DOCUMENT NUMBER: 135:209984
TITLE: Manufacture of large crystals of δ-endotoxins with Bacillus thuringiensis by increasing gene copy number
INVENTOR(S): Adams, Lee Fremont; Thomas, Michael David; Sloma, Alan P.; **Widner, William R.**
PATENT ASSIGNEE(S): Valant Biosciences, Inc., USA; Libertyville, Inc.
SOURCE: U.S., 19 pp., Cont.-in-part of U. S. Ser. No. 92,338, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 4
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6280720	B1	20010828	US 1994-274608	19940713
IN 178296	A	19970322	IN 1994-MA623	19940712
CA 2167178	AA	19950126	CA 1994-2167178	19940714
ZA 9405138	A	19950223	ZA 1994-5138	19940714

CZ 290015	B6	20020515	CZ 1996-96	19940714
ES 2196030	T3	20031216	ES 1994-922566	19940714
US 6280721	B1	20010828	US 1995-377891	19950125
US 6303382	B1	20011016	US 1996-771190	19961220
US 6270760	B1	20010807	US 1997-872571	19970610
US 5955367	A	19990921	US 1998-60288	19980414
PRIORITY APPLN. INFO.:			US 1993-92338	B2 19930715
			DK 1989-6396	A 19891218
			US 1992-853701	B2 19920526
			US 1994-274608	A2 19940713
			US 1995-377892	B1 19950125
			US 1995-378236	B1 19950125

AB The invention relates to a method for producing an integrant(s) of Bacillus thuringiensis which produces a larger quantity of a crystal δ-endotoxin with a greater pesticidal activity than the crystal delta-endotoxin produced by the corresponding parental strain. The crystal δ-endotoxin produced by the integrant Bacillus thuringiensis will have an activity directed towards the same pest(s) as its parent Bacillus thuringiensis crystal δ-endotoxin. The invention further relates to such integrants, compns. comprising such integrants, as well as methods for controlling a pest(s) using these compns. Integration is achieved by transformation with a plasmid that does not carry a replicon that functions in B. thuringiensis but that does carry sequence that will direct efficient integration of the plasmid into the host chromosome. Use of the cryIII and **cryIIIA** genes is demonstrated. Parasporal crystals from integrant hosts were up to twice as long and 30% wider than crystals from parental strains. LC50's for the δ-endotoxins of the integrants were 3-8-fold lower than those of the parental strains.

REFERENCE COUNT: 47 THERE ARE 47 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L14 ANSWER 5 OF 6 HCAPLUS COPYRIGHT 2006 ACS on STN

ACCESSION NUMBER: 1999:566201 HCAPLUS

DOCUMENT NUMBER: 131:180803

TITLE: Nucleic acid vectors for recombinant production of heterologous proteins in a Bacillus cell

INVENTOR(S): **Widner, William**; Sloma, Alan; Thomas, Michael D.

PATENT ASSIGNEE(S): Novo Nordisk Biotech, Inc., USA

SOURCE: PCT Int. Appl., 90 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9943835	A2	19990902	WO 1999-US4360	19990226
WO 9943835	A3	19991125		
W: AL, AU, BB, BG, BR, CA, CN, CU, CZ, EE, GE, HU, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LU, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 5955310	A	19990921	US 1998-31442	19980226
AU 9929756	A1	19990915	AU 1999-29756	19990226
EP 1056873	A2	20001206	EP 1999-911012	19990226
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, NL, SE, PT, IE, FI				
JP 2002504379	T2	20020212	JP 2000-533574	19990226
CN 1510145	A	20040707	CN 2003-2003158121	19990226
US 2003170876	A1	20030911	US 2001-834271	20010412

PRIORITY APPLN. INFO.: US 1998-31442 A 19980226
US 1999-256377 B3 19990224
WO 1999-US4360 W 19990226

AB The present invention relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a tandem promoter in which each promoter sequence of the tandem promoter is operably linked to a single copy of a nucleic acid sequence encoding the polypeptide, and alternatively also (ii) an mRNA processing/stabilizing sequence located downstream of the tandem promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to methods for producing a polypeptide, comprising: (a) cultivating a Bacillus host cell in a medium conducive for the production of the polypeptide, wherein the Bacillus cell comprises a nucleic acid construct comprising (i) a "consensus" promoter having the sequence TTGACA for the "-35" region and TATAAT for the "-10" region operably linked to a single copy of a nucleic acid sequence encoding the polypeptide and (ii) an mRNA processing/stabilizing sequence located downstream of the "consensus" promoter and upstream of the nucleic acid sequence encoding the polypeptide; and (b) isolating the polypeptide from the cultivation medium. Random promoters are created by placing promoters such as amyQ and amyL upstream of the **cryIIIA** promoter and its mRNA stabilizing sequence. Alternatively, "consensus" amyQ promoters are created with the **cryIIIA** mRNA stabilizing sequence, as well as tandom copies of a single promoter such as the short consensus amyQ dimer and trimer promoters. All of these approaches lead to significantly higher levels of SAVINASE gene expression (up to 620%) in Bacillus cells when compared to the levels obtained using single promoters such as amyQ and amyL.

L14 ANSWER 6 OF 6 BIOTECHDS COPYRIGHT 2006 THE THOMSON CORP. on STN
DUPLICATE 2
ACCESSION NUMBER: 1995-04013 BIOTECHDS
TITLE: Methods for producing large Bacillus thuringiensis crystals; crystal protein overproduction by integration via homologous recombination and marker rescue, for use in biological control agent strain improvement
AUTHOR: Adams L F; **Thomas M D**; Sloma A P; **Widner W R**
PATENT ASSIGNEE: Novo-Nordisk-Entotech; Novo-Nordisk-Biotech
PATENT INFO: WO 9502695 26 Jan 1995
APPLICATION INFO: WO 1994-US7955 14 Jul 1994
PRIORITY INFO: US 1993-92338 15 Jul 1993
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: WPI: 1995-067339 [09]

AB A new integrant of Bacillus thuringiensis produces an increased quantity of delta-endotoxin crystal protein with improved pesticidal activity against the same pest as the parent, and is produced by homologous recombination using a vector with a homologous region and a selectable marker (e.g. antibiotic-resistance) in the presence of increasing amounts of a selective agent for gene amplification. The toxin is encoded by the cryI, cryII, cryIII, cryIV, cryV or cryVI gene, preferably **cryIIIA**. The integrant may be B. thuringiensis EMCC 0082 (NRRL B-21106), EMCC 0083 (NRRL B-21107), EMCC 0115 (NRRL B-21286) or EMCC 00116 (NRRL B-21287). The toxin gene is from B. thuringiensis subsp. kurstaki, aizawai (preferred), galleriae, entomocidus, tenebrionis (preferred), thuringiensis, alesti, canadiensis, darmstadiensis, dendrolimus, finitimus, kenyae, morrisoni, subtoxicus, toumanoffi or israelensis. A 2-vector double selectable marker rescue system may be used for recombination. The host is incubated at 37 deg. The crystal protein-producing strain is active against Coleoptera or Lepidoptera

insect pests. (44pp)

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     (FILE 'HOME' ENTERED AT 14:23:15 ON 23 FEB 2006)

     FILE 'MEDLINE, EMBASE, BIOSIS, BIOTECHDS, SCISEARCH, HCAPLUS, NTIS,
     LIFESCI' ENTERED AT 14:23:47 ON 23 FEB 2006
L1        395086 S BACILLUS
L2           560 S CRYIIIA
L3           516 S L1 AND L2
L4           635 S "TTGACA" OR "TATAAT"
L5            13 S L3 AND L4
L6             6 DUP REM L5 (7 DUPLICATES REMOVED)
L7             7 S L2 AND "CONSENSUS PROMOTER"
L8             5 DUP REM L7 (2 DUPLICATES REMOVED)
                 E WIDNER W/AU
L9           119 S E3-E9
                 E SLOMA A/AU
L10          124 S E3
                 E THOMAS M D/AU
L11          404 S E3
L12          632 S L9 OR L10 OR L11
L13            8 S L2 AND L12
L14            6 DUP REM L13 (2 DUPLICATES REMOVED)
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	Issue Date	Pages	Document ID	Title
1	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
2	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
3	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
4	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
5	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
6	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell

	Issue Date	Pages	Document ID	Title
1	20001031	35	US 6140104 A	Nucleotide sequences for the control of the expression of DNA sequences in a cell host

	Issue Date	Pages	Document ID	Title
1	20060223	32	US 20060040281 A1	Polyhydroxybutyrate polymerase
2	20051208	21	US 20050272695 A1	Fast dissolving dried hyaluronic acid product
3	20051201	21	US 20050267068 A1	Dried and agglomerated hyaluronic acid product
4	20051103	114	US 20050246798 A1	Novel glyphosate-N-acetyltransferase (GAT) genes
5	20051006	94	US 20050221446 A1	Methods for producing hyaluronic acid in a Bacillus cell
6	20050901	47	US 20050191628 A1	Antibiotics based upon bacteriophage lysis proteins
7	20050414	55	US 20050079617 A1	Glucose transport mutants for production of biomaterial
8	20050324	34	US 20050064565 A1	POLYHYDROXYBUTYRATE POLYMERASE
9	20040819	16	US 20040162417 A1	Protease, a gene therefor and the use thereof
10	20040429	86	US 20040082770 A1	Novel glyphosate N-acetyltransferase (GAT) genes
11	20040226	108	US 20040038262 A1	Ribulose 1,5-bisphosphate carboxylase/oxygenase polypeptides and related polynucleotides
12	20040205	218	US 20040023205 A1	Method of recovering a nucleic acid encoding a proteinaceous binding domain which binds a target material

	Issue Date	Pages	Document ID	Title
13	20040108	218	US 20040005539 A1	Nucleic acids, genetic constructs, and library of nucleic acids encoding fusion proteins
14	20031218	61	US 20030232406 A1	Bacterial strains which overproduce riboflavin
15	20031204	114	US 20030225249 A1	32-kDa protein derived from mycobacterium tuberculosis and related peptides
16	20031127	197	US 20030219886 A1	Directed evolution of novel binding proteins
17	20031127	224	US 20030219722 A1	Fusion proteins, modified filamentous bacteriophage, and populations or libraries of same
18	20031002	22	US 20030186380 A1	Methods for producing secreted polypeptides having L-asparaginase activity
19	20030918	142	US 20030175902 A1	Methods for producing hyaluronan in a recombinant host cell
20	20030911	57	US 20030170876 A1	Methods for producing a polypeptide in a bacillus cell
21	20030619	43	US 20030115627 A1	Coniothyrium minitans beta-(1,3) exoglucanase gene cbeg1
22	20030619	190	US 20030113717 A1	Directed evolution of novel binding proteins
23	20030501	139	US 20030083480 A1	Novel glyphosate N-acetyl transferase (GAT) genes

24	20021017	203	US 2002015088 1 A1	Directed evolution of novel binding proteins

	Issue Date	Pages	Document ID	Title
25	20020912	33	US 20020127240 A1	Leptospiral rare outer membrane proteins
26	20060103	70	US 6982314 B2	Lawsonia intracellularis proteins, and related methods and materials
27	20051227	303	US 6979538 B2	Directed evolution of novel binding proteins
28	20050419	36	US 6881560 B2	Polyhydroxybutyrate polymerase
29	20050111	56	US 6841376 B2	Bistable genetic toggle switch
30	20040511	42	US 6734344 B2	Coniothyrium minitans .beta.-(1,3) exoglucanase gene cbeg 1
31	20040413	217	US 6720488 B2	Transgenic maize seed and method for controlling insect pests
32	20040302	33	US 6699482 B2	Leptospira rare outer membrane proteins
33	20030422	60	US 6551813 B1	Nutrient medium for bacterial strains which overproduce riboflavin
34	20030311	117	US 6531138 B1	Recombinant polypeptides and peptides, nucleic acids coding for the same and use of these polypetides and peptides in the diagnostic of tuberculosis
35	20030304	35	US 6528706 B1	Polyhydroxybutyrate polymerase
36	20020521	32	US 6392126 B1	Adenosine deaminase homologues and uses thereof

37	20011127	109	US 6322995 B1	Riboflavin production

	Issue Date	Pages	Document ID	Title
38	20011106	36	US 6313376 B1	Maize aquaporins and uses thereof
39	20010918	11	US 6291230 B1	Galk promoter
40	20010724	36	US 6265636 B1	Pyruvate dehydrogenase kinase polynucleotides, polypeptides and uses thereof
41	20010703	54	US 6255076 B1	Methods for producing a polypeptide in a Bacillus cell
42	20010626	21	US 6252140 B1	Promoters from chlorella virus genes providing for expression of genes in prokaryotic and eukaryotic hosts
43	19990720	63	US 5925538 A	Bacterial strains which overproduce riboflavin
44	19990629	119	US 5916558 A	Recombinant polypeptides and peptides, nucleic acids coding for the same and use of these polypeptides and peptides in the diagnostic of tuberculosis
45	19981201	69	US 5843426 A	Salmonella vaccines
46	19981117	62	US 5837528 A	Bacterial strains which overproduce riboflavin
47	19981117	181	US 5837500 A	Directed evolution of novel binding proteins
48	19981117	33	US 5837263 A	Leptospira membrane proteins
49	19980825	25	US 5798235 A	Gene encoding bacterial acetoacetylco a reductase

50	19980811	21	US 5792924 A	Biologically safe plant transformation system

	Issue Date	Pages	Document ID	Title
51	19980324	14	US 5731151 A	Regulator of contact-mediated hemolysin
52	19970902	32	US 5663063 A	Method for producing polyester biopolymers
53	19970826	24	US 5661026 A	Gene encoding bacterial beta-ketothiolase
54	19961105	176	US 5571698 A	Directed evolution of novel binding proteins
55	19960709	33	US 5534432 A	Polyhydroxybutyrate polymerase
56	19960430	25	US 5512669 A	Gene encoding bacterial acetoacetyl-COA reductase
57	19960116	34	US 5484718 A	Nodulation gene promoter
58	19960109	21	US 5482852 A	Biologically safe plant transformation system
59	19950404	199	US 5403484 A	Viruses expressing chimeric binding proteins
60	19931005	33	US 5250430 A	Polyhydroxyalkanoate polymerase
61	19930914	32	US 5245023 A	Method for producing novel polyester biopolymers
62	19930720	25	US 5229279 A	Method for producing novel polyester biopolymers
63	19930706	19	US 5225341 A	Biologically safe plant transformation system using a Ds transposon
64	19921215	20	US 5171673 A	Expression of heterologous DNA using the bacillus coagulans amylase gene

65	19910122	13	US 4987078 A	Plasmid vectors for expression in Escherichia coli and/or Bacillus subtilis

	Issue Date	Pages	Document ID	Title
66	19900417	36	US 4918006 A	Gene coding for insecticidal crystal protein
67	19880906	42	US 4769327 A	Secretion vector

	L #	Hits	Search Text
1	L1	1	"6852518".pn.
2	L2	4	"GST-40"
3	L3	1	l1 and l2
4	L4	41401	bacillus
5	L5	365	cryIIIA
6	L6	314	l4 same l5
7	L7	495	"TATAAT" or "TTGACA"
8	L8	6	l6 same l7
9	L9	3016	"downstream region"
10	L10	1	l6 same l9
11	L11	411391	WIDNER SLOMA THOMAS
12	L12	120	l7 and l11
13	L13	67	l4 and l12